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Is It or Isn't It: The Importance of Visual Classification in Microplastic Characterization.

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Abstract

Microplastics are a diverse category of pollutants, comprising a range of constituent polymers modified by varying quantities of additives and sorbed pollutants, and exhibiting a range of morphologies, sizes, and visual properties. This diversity, as well as their microscopic size range, presents numerous barriers to identification and enumeration. These issues are addressed with the application of physical and chemical analytical procedures; however, these present new problems associated with researcher training, facility availability and cost, especially for large-scale monitoring programs. Perhaps more importantly, the classifications and nomenclature used by individual researchers to describe microplastics remains inconsistent. In addition to reducing comparability between studies, this limits the conclusions that may be drawn regarding plastic sources and potential environmental impacts. Additionally, where particle morphology data is presented, it is often separate from information on polymer distribution. In establishing a more rigorous and standardized visual identification procedure, it is possible to improve the targeting of complex analytical techniques and improve the standards by which we monitor and record microplastic contamination. Here we present a simple and effective protocol to enable consistent visual processing of samples with an aim to contribute to a higher degree of standardization within the microplastic scientific community. This protocol will not eliminate the need for non-

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subjective methods to verify plastic objects, but it will standardize the criteria by which suspected plastic items are identified and reduce the costs associated with further analysis.
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Introduction

Over the past decade, there have been an increasing number of research articles published on the presence of microplastics in environmental samples.[1–5] Whilst the aims, methodology and findings of these studies are highly variable, the identification of microplastics remains universally important. Unlike other environmental pollutants such as macrolitter which may be visually identified with relative confidence,[6,7] or heavy metals that can be measured via assays,[8,9] the relative size, varied morphologies, and inert chemistry of microplastic debris present a range of challenges for identification by researchers. As a result, many researchers have focused on establishing robust methodologies for the characterization of the particles recovered;[10,11] these techniques attempt to confirm that an object is plastic or to identify the specific polymer present. While this approach is essential to accurately assess the abundance of microplastics, this level of rigor and consistency has not been applied to the visual analysis and the classification of microplastics. Further, visual analysis of samples is essentially ubiquitous throughout microplastic research, whether employed during manual separation, following initial separation or in support of further characterization,[10–13] the lack of standardization in the nomenclature of microplastic research reduces comparability between studies, resulting in challenges to monitoring, mitigation, and policy. Additionally, visual analysis classification is essential in supporting the various methods for characterization and can assist in reducing potential shortcomings of these methods.

Characterization

As mentioned above, a range of physical and chemical approaches to polymer characterization are routinely employed to determine the presence and/or polymer structure of plastics in a sample;[12,13] the use of which has revealed that investigators are highly variable in their ability to identify plastics. Studies of microplastic contamination in both biota and environmental samples have reported positive misidentification rates of between 20 and 98.6% versus actual values;[10,14,15,16] additionally, some plastics, such as clear fibers have a high probability of accidental exclusion.[14] Despite the high probability of misidentification, in many studies only a proportion of particles identified as microplastic have been subject to

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confirmatory analysis, sometimes as low as 5–10 %,[17] with the analyzed portion restricted by factors such as total sample size, particle size, processing time (throughput), analytical costs ,and the availability of facilities.

Chemical Characterization of Plastic Polymers

The most commonly applied analytical methods to determine both the presence of plastics and the range of polymers recovered are IR spectroscopic techniques such as Fourier transform infrared (FT-IR) and Raman spectroscopy.[10,12] Whilst these are able to give a great deal of detail regarding the composition of the suspected plastics, accurate interpretation requires a high degree of experience on the part of the researcher. Challenges are presented by sample preparation (e.g., compatible filter materials), the sample interface with the instrumentation and low-quality spectra arising from particles covered with biofilms, heavily degraded polymers or those containing additives or adsorbed contaminants. Changes in the chemical structure of the polymer alter the height and width of the peaks in the spectrum as weaker bonds are broken and remaining bonds are deformed.[18] Further obstacles are presented by the presence of regenerated cellulose, such as Rayon and Modal, the spectra of which may be easily confused with those of natural cellulosic materials. Recently, the development of semi-automated imaging spectroscopy systems has somewhat reduced the need for the visual pre-assessment and isolation of microplastics from sample matrices;[19] however, this has increased the cost of equipment and training, as well as the level of prerequisite sub-sampling increasing the time required to process samples. Moreover, visual inspection of photographed filters may still be necessary to quality assure and control the output of analytical software.

Less commonly, but increasingly used, pyrolysis and thermal desorption gas chromatography–mass spectrometry (py-GC-MS; TED-GC-MS) represent important alternatives to IR spectroscopy.[12,19,20] Whilst these methods have the additional advantage of revealing the mass of plastic material in a sample, no information regarding the morphology of the particle is given and the volume of sample that may be processed is limited.[12,20] While spectrometry and spectroscopy characterize polymer structure, visual classification also allows for accurate and consistent recording of particle morphologies, data essential in identifying potential sources of microplastics, their behavior and environmental fate, and interaction with biota.[21–23]

Physical Characterization of Plastic Polymers

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In addition to spectrometry and spectroscopy techniques, a range of physical properties are used to separate potential microplastics from sample matrices and reduce the likelihood of misidentification via the exclusion of non-plastic particles. Many of these properties are also useful in separating particles from environmental matrices, resulting in dual purpose processing such as density separation, digestion, and magnetic extraction. Density separation, which utilizes solutions with a high specific gravity, is amongst the most commonly applied methods used to separate plastic, and other low-density materials from heavier sample matrices, such as benthic sediments.[11,13] Sequential density extraction procedures may also be used to isolate particles based upon their behavior in different aqueous media; for example, polypropylene, polyethene and ethyl vinyl acetate are polymers with specific densities <0.98 g cm^{-3} can be separated using freshwater alone. Separation using saturated salt (sodium chloride, NaCl) solution (1.2 g cm^{-3}) will also suspend polystyrene, acrylics, polyamides and polymethylmethacrylate. Denser liquids, such as sodium iodide, Na(I), 1.8 g cm^{-3} are required for separating more dense particles such as polyesters. Limitations of density separation include the inability of salts to extract the densest polymers, such as Teflon, from sediments and the influence of biofilms and other fouling material on net polymer density.[24]

Where density separation removes plastics from samples, digestion is commonly used to remove non-plastic organic material that may otherwise negatively affect the isolation and identification of microplastics.[13] Digestion to reduce or remove organic components can significantly improve analysis, and is considered essential for sample types with high organic content, particularly in the case visual or IR spectroscopic techniques will later be employed. Some acidic and alkaline procedures have, however, been shown to impact microplastic particles, potentially leading to complete degradation.[25] Less damaging processes, such as enzymatic digestion, can be a suitable alternative but can also be very time consuming for samples containing several different types of organic material (such as cellulose, chitin, proteins, lipids). Even for the most effective organic matter removal techniques, some natural materials may remain.[13] Hence, visual identification techniques may still be required to separate plastic from natural materials.

Recently it has also been demonstrated that plastics can be magnetically extracted from samples by taking advantage of their hydrophobic surface properties.[26] In creating hydrophobic iron (Fe) nanoparticles which bind to plastics, Grbic and colleagues[26] were able to use magnetic recovery of microplastic with recovery rates exceeding 78%. This method appears to be useful for cleaner matrices such as drinking water, or those post-density separations.

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Other physical techniques for the characterization of microplastics focus on individual particles rather than their isolation from larger matrices and may be used in combination with the methods above.[27] These methods utilize a number of properties of the plastic polymer, such as lithophilic properties and melting point. Lipophilic dyes to stain samples prior to observation under a fluorescence microscope has been used in the identification of commonly overlooked plastics, particularly white or clear items or those in the smaller size range (approx. <100 μm), as well as the enumeration of plastic particles in semi-clean samples, when plastics may be masked by non-plastic solids.[27–29] These stains, such as Nile Red, take advantage of the hydrophobic properties of plastics and have been suggested as an alternative to the conventional microscope and spectroscopic methods.[28] However, the ease of analysis is limited by the potential co-staining of natural material such as lipids and other organic matter. To compensate for this pre-digestion, choice and stain solvent and fluorescence excitation wavelength must be carefully considered.

The identity of suspected plastics has also been confirmed using the "hot-needle" test or hot-stage melting.[30,31] The hot-needle test utilizes the application of heat to induce melting in the suspected particle. During hot-stage melting, potential microplastics are placed on a heated stage under a microscope. By progressively increasing the temperature of the stage, it is possible to determine a plastic constituent polymer by its melting point. Most polymers have a melting point between 110ºC low-density polyethylene (LDPE) and 330ºC Teflon; where the exact temperature range varies depending on the level of crosslinking between polymer chains. By noting the temperature at which a particle melts it is possible to confirm with greater reliability the presence of plastic and, potentially, its constituent polymer. However, this process is slow, is primarily suitable for thermoset polymers rather than thermosetting polymers,[32] and may be complicated by polymers with similar melt temperature, natural rubbers and other natural materials which exhibit similar phase transition behavior.

As with the techniques for polymer characterization outlined above, the varied methods for microplastic separation and organic matter removal are subject to drawbacks which make them unsuitable for use independently. An understanding of the visual characteristics of plastics remains essential to assist in confirming abnormal observations, reducing the potential for false-positive or negative classification of particles, and providing information regarding particle origin and potential impacts. Effective, global guidelines for the classification of microplastics will assist in increasing the reliability of analyses and improving assessments of spatial and temporal microplastic trends in all environmental

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matrices. The aim of this manuscript is to present a method to aid in visual identification and descriptions of particles found in samples, to provide researchers with a guide to identifying particles and highlight methods to exclude non-plastic items.

Classifying Microplastics

As shown above, visual identification of microplastic may be affected by subjective differences between researchers, resulting in inconsistencies between laboratories and even individual analysts. Below we outline a robust identification process to facilitate improved visual identification processes and increase harmonization among studies. Following initial separation or isolation of potential plastics, particles are typically observed under a microscope. At this stage, individual particles should be assessed based on a hierarchy of physical and visual characteristics to discount non-plastic materials and classify suspected microplastics. Within this hierarchy, three descriptive categories can be used to aid in visual descriptions of microplastics: morphology (size, shape, and texture), optical properties (color, reflectivity, and birefringence) and behavior (flexibility, density, etc.). Whilst the classification of particles typically occurs after identification as a suspected microplastic, this process will be reviewed first as it establishes important terminologies and particle categories that will be used in the visual identification key presented later.

Identifying and Classifying Based Upon Morphology

Particle size. Microplastics can be grouped into size categories. Pellets and other similar raw plastic production materials typically have very different structure and function to the fragments, fibers and granular particles that are <1 mm. Unfortunately, there is inconsistency in how researchers record size. Measuring fibers in their longest dimension categorizes them as large microplastics or even mesoplastics, but by their diameter, they are within the smallest microplastic size classes. Feret's diameter has been suggested as the most appropriate measurement, as it refers to the length of the shortest line joining two points of an object outline.[33,34] What is clear is that researchers should report particles sizes more explicitly than simply less than or more than 5 mm. Increasing the number of size categories, if sizes must be grouped, allows for interpretation on a much wider scale than when reported in few bins[35]. Other alternatives include describing particles by their two-dimensional surface area, or three-dimensional volume. This helps to account for large discrepancies in particle length and width, such as in the case of fibers, but may attenuate particle data related to morphology that is also highly relevant for determining environmental behavior and fate.

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Particle Shape. Microplastic particles exhibit highly heterogeneous morphologies and have been described using an equally diverse set of terminologies, including spheres, pellets, grains, beads, granules, filaments, fibers, lines, films, foams, expanded polystyrene, and fragments. Recording particle morphology is of value when attempting to identify potential sources or inferring environmental interaction, such as particle behavior in environmental systems and the potential for ingestion by biota. For example, fibers may derive from textiles or rope and their irregular shape may influence the way in which they sink in aquatic environments. Presently, however, there is no standardized nomenclature for classifying particles by morphology and thus, recording is often subjective and comparisons between different studies are complicated. Here we separate existing microplastic morphologies into three core groups and suggest appropriate sub-categories in the event that more detailed recording is necessary (Table I) – for example, in the case of identifying specific point sources such as microbeads from personal care products or films from agricultural land.

Beads. Also termed pellets, grains, and spheres, beads are typically primary microplastics – plastics which are specifically engineered to be small in size (i.e., in the microsize range). There are a range of structural shapes in this category particularly with reference to pre-production resin pellets (nurdles) that may be spherical, ovoid, or cylindrical granules, microbeads from personal care products which may appear as granules with less regular particle surface, or highly spherical beads such as those used in water treatment processes.[36] Helm[22] suggested distinguishing between spheres and pellets due to differences in potential sources. Spheres are rounded and smooth in surface texture and are typically smaller in size than pellets (~100 μm to 1 mm versus ~3–5 mm).[37] The sub-category of spheres may also include hemispheres, resulting from the fracture of spheres during the manufacturing process or due to breakage from weathering or sample processing. We recommend that researchers use the lowest level of categorization possible to aid comparisons between studies.

Fibers. Also described as filaments, fibers often account for the highest proportion of particles identified in environmental samples. Fibers are characterized by a length that is significantly longer than the width. Widths typically below 50–75 μm and more commonly around 10–30 μm, with longer lengths that can be up to several mm or even cm. Extruded fibers are typically identified by their homogenous thickness throughout their length;[11] however, care must be taken not to exclude fibers of alternative origin, such as twisted film ropes. Typically, plastic fibers will have a smooth surface; however, textile production methods sometimes induce “fibrilization” or the formation of smaller fibers (fibrils) on the

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fabric surface, which results in a less even structure.[38] Fibers may also be present in bundles, which are several fibers tightly wound together in a knot-like formation. Fiber bundles should be teased apart and counted as individual fibers whenever possible, especially when bundles contain fibers of dissimilar appearance. Fiber bundles should only be counted as such when there are >20 fibers of consistent appearance, or when separation would cause breakage leading to a greater number of individual fibers than were initially present in the sample.[37] Fiber length can be measured and reported in the following ways: (i) total length i.e., full length traced using software; (ii) longest visual dimension with a straight line; and (iii) maximum Feret's diameter. As researchers can use different measurement procedures depending on the context of their individual studies, we recommend researcher report as many measurements as possible or be explicit about the measurement approach employed.

Fragments. Predominantly formed by the degradation of larger materials,[39–41] the breakdown of larger plastic items into microplastic particles is not yet well defined, but likely occurs due to a very wide range of processes that relates to polymer type and associated structure, method of polymerization, occurrence of additives, method(s) of degradation (physical, chemical, biological) and environmental conditions. This results in a highly diverse category of particles, which also encompasses sub-categories such as foams and films. Fragments may be separated from beads by their relative angularity (Figure 1). Films are fragmented materials in which two dimensions are significantly greater than the third, where the latter may be just a few micrometers. Films are often described as being more flexible than fragments and may be folded or creased while remaining somewhat resistant to breakage;[33] however, certainly, polymer-based paints may be relatively brittle. Foams are fragments from materials to which a blowing agent is introduced during the setting process, and includes expanded polystyrene (EPS), expanded polyvinyl chloride (EPVC, or Foamex), and similar. Foams can be squashed when physically handled, but spring back into shape.[37] They are categorized as fragments when it is apparent that they are derived from a larger plastic items, and not in the case of complete EPS beads, for example. We recommend that researchers use the lowest level of categorization possible to aid comparisons between studies.

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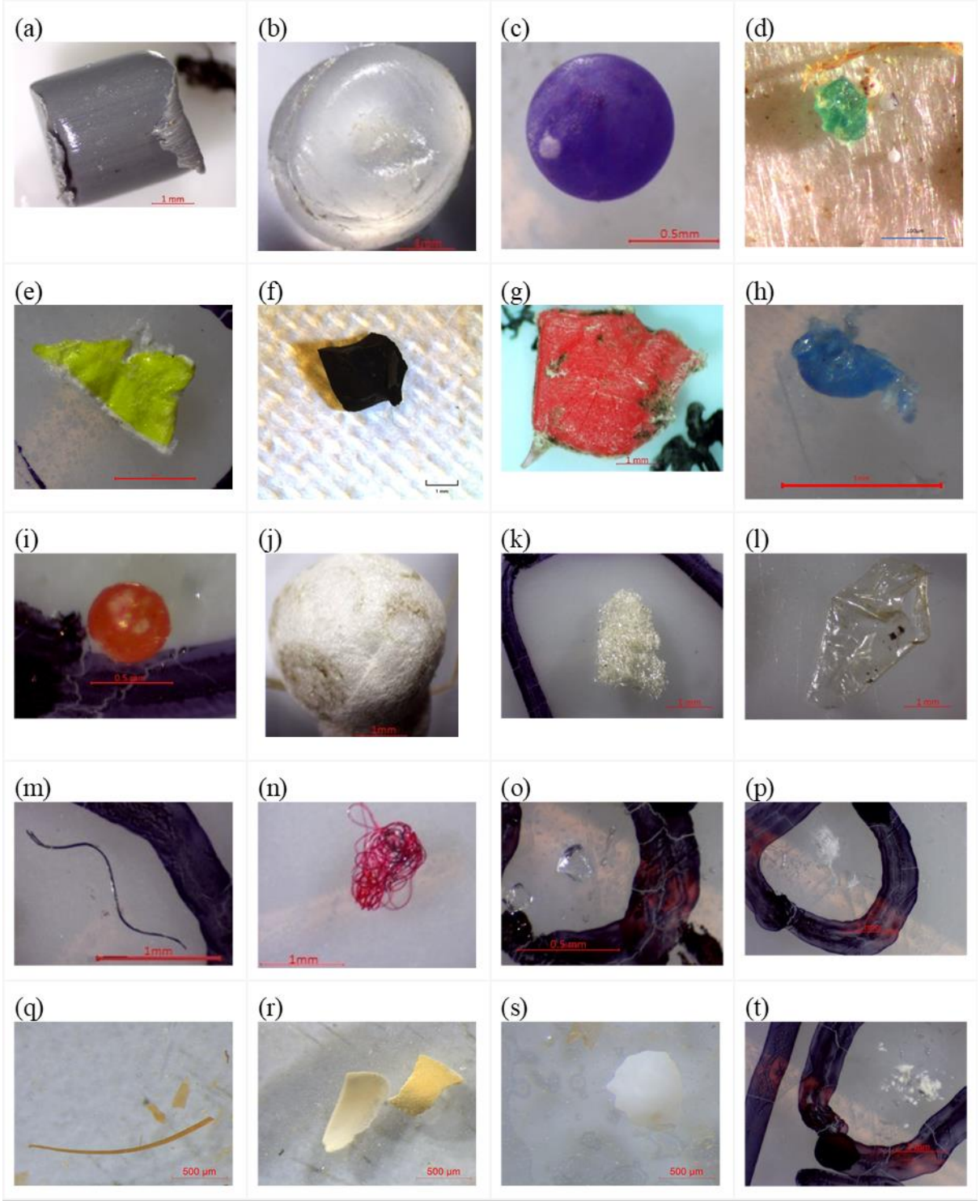

Figure 1. Library of visually identified particles from samples composing of (a–n) confirmed microplastics and (m–t) non-plastic material. Images marked with asterisks (*) are from the San Francisco Estuary Institute, 2019; # from Rochman et al.[37]). (a) Resin pellet*; (b) oval or disk pellet#; (c) bead; (d) plastic grain; (e) yellow angular fragments; (f) subangular fragment; (g) rounded fragment*; (h) blue sub-rounded fragment; (i) bead; (j) Styrofoam (EPS) bead#; (k) Foam; (l) film; (m) fiber; (n) fiber bundle; (o) sand grain; (p) cellulose, plant material or

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toilet paper; (q) KOH treated bone; (r) KOH treated shell fragment; (s) KOH treated otolith; (t) horn which has crumbled under pressure.

Table I. Microplastic terms and sub-categories recommended for describing and categorizing morphologies.

	Sub-categories	Short description
Bead	Sphere (hemisphere), grain, granule, pellet, nurdle, bead, EPS balls, ball	May be spherical or granular. Does not have any broken edges that indicate it has originated from a larger particle. Shows signs of having been specifically engineered to be of a particular shape and size.
Fiber	Filament, string, fibrous, fiber bundle	Long fibrous material that has a length substantially longer than its width.
Fragment	Film, foam	May have smooth or angular edges, may be flat or angular but generally has the appearance of having broken down from a piece of larger debris.

Surface Roughness. The process of molding or extrusion and the mix of fillers applied to a polymer result in a highly diverse range of surface textures. In relatively undegraded polymers, the surface texture will be uniform across the polymer surface. Following extended environmental exposure, embrittlement, biofouling, and bleaching may result in an uneven, pitted appearance.[42] In the event of a highly degraded or biofouled particle, the researcher may attempt to break or clean the surface to reveal a previously unexposed surface more representative of the original material. We recommend that researchers attempt to characterize surface roughness where possible.

Identifying and Classifying Based Upon Optical Properties

Color. Homogeneous color is suggested to be indicative of plastic particles, in particular for fragments.[11,43] Despite this, it is important to note that exceptions do exist, especially in the case of particles that are composed of multiple colored sections or as a result of leaching following digestion protocols. Color descriptions should be addressed with great care due to weathering of in situ particles, color blindness and different color perception amongst researchers. This may be further complicated through differences in ambient lighting or

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microscope light source. A possible solution to this problem could be to use a color meter to impartially identify specific particle color, however to our knowledge this has not been tested on small plastic particles and may be limited to larger particles only.

Using broad color classifications is not sufficient to indicate particle similarity, given the range of shades available within a single category (e.g., navy, turquoise, sky blue, cyan as blue). Additionally, the full range of reagents used within microplastic studies has not yet been specifically tested for the preservation of plastic color. In some cases, leaching of color has been observed after processing microplastic samples, such as potassium hydroxide (KOH) digestion affecting a pink polyethylene terephthalate fiber.[44] Despite this, it is still recommended to record particle color during visual assessment. Whilst source derivation is not likely possible based on color alone, recording color may help to identify broad trends, such as ingestion preference.[45,46] It may also be important to assess airborne contamination of samples from clothing or sampling apparatus, for example. To reduce bias among researchers using visual identification to classify color of microplastics, we recommend recording colors to the level of secondary colors (red, orange, yellow, green, blue, violet) as well as including black, white, and clear.

Reflective Properties. In addition to influencing the surface texture and color of the plastic product, the polymer structure, manufacturing processes (such as molding and extrusion) and the presence of fillers can influence both the light transmitting and reflective properties of plastics. Plastics exhibit a range of structures depending on the level of branching and interconnection of the polymer chain, which affect the properties of the polymers.[32] Crystallinity of the polymer structure influences the passage of light; plastics with amorphous structures transmit light, whereas semi-crystalline structures scatter light, resulting in increased opacity.[47] Beyond the passage of light through the polymer, manufacturing processes have developed a range of methods by which to affect the gloss, luster and surface roughness of the end product. During molding, crystalline vs amorphous polymer structure, processing temperature, rate of injection and use of reinforcements influence the roughness and gloss of the final product.[48–50] Stresses exerted during the extrusion of polymer fibers result in consistent crystalline properties (anisotropy) which may be observed and measured under polarized light; known as birefringence. Observation of birefringence, gloss, and fine surface texture are regularly cited as factors used to identify plastics; however, care must be taken to exclude regenerated natural materials, other manufactured products, and silicates. Researchers should first determine whether fibers are isotropic or anisotropic by rotating the orientation of the polarizing filter by 90°. Anisotropic,

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will change in appearance as the polarization plane is rotated. They should note the refractive indices of samples observed when the polarizing lens is parallel with and perpendicular to the studied item, and (if appropriate) determine the resulting degree of birefringence. These may then be compared to reference sample known values.

Identifying and Classifying Based Upon Physical Behavior

Testing the physical properties of a suspected microplastic particle can provide substantial insight into its possible composition. This can be performed by physically handling the particle using microforceps or dissecting needles. The degree of elasticity is particularly important, where plastic particle types are often yielding when squeezed or prodded, a property typically conferred by the presence of plasticizers[47] or highly cross-linked elastomers.[32] This can be a useful technique to separate out sand particles, for example, from clear plastic granules, such as those found in personal care products. This texture can be described as yielding but not malleable, where particles return to their original shape after pressure is released. Rubbery particles will exhibit a higher degree of elasticity than more rigid polymer types. We recommend that researchers physically manipulate particles (>200 μm) to aid in visual identification.

Particles that are easily broken with minimal force are more likely to be derived from organic material, where plastic particles often have a higher degree of integrity. However, it is important to note that highly weathered plastics may also be brittle, and so no sole characteristic should be relied upon for visual identification. Thin films that fragment upon contact are more likely to be algal films or dried, partially dissolved organic material. Additionally, some seed casings or insect carapaces can appear as small, shiny, spherical beads or fragments but break easily when pressure is applied. During physical handling of particles, through squeezing and prodding, the particle texture may also be inferred through sound. This is particularly relevant for mineral particles, the hardness of which generates a "crunching" when pressure is applied.

Utilizing a secondary density check may also help to identify suspected microplastic particles. By placing the particle into aqueous media with different densities, it may be possible to separate plastic particles from some natural materials. For example, glass beads used in reflective road paint can appear similar to rigid, clear spherical plastic beads but glass beads will not float in salt solutions with densities less than approximately 2.5 g cm^{-3}.

Key for the Classification of Suspected Microplastic Particles

DOI: 10.1177/0003702820930733

By sequentially observing features outlined in the section above it will be possible to reduce inter-researcher subjectivity and improve the consistency and comparability of results. We suggest that researchers follow the hierarchy outlined in the key below as well as the accompanying flow chart (Figure S1, Supplemental Material). By using the key, our aim is to harmonize the visual identification of microplastics, as well as streamlining pre-selection for polymer characterization. Categories with known commonly confused or misidentified items are marked with an " * " and readers are advised to read the appropriate exclusion parameters found later in this document. Particle size adds complexity to the challenge of identification especially in terms of physical handling, which can be challenging for particles <200 μm. Therefore, this workflow targets particles, which can be easily handled.

I. When pressure is applied, the suspected particles have a:

Solid, inflexible, and firm structure	2
Flexible, soft, or malleable structure	14

II. The overall shape is:

Clearly defined	3
Fragmented	10

III. Is the shape cylindrical or spherical (includes oval/hemisphere)?

Cylindrical	4
Spherical	5

IV. This may be classified as a pre-production resin pellet pending confirmatory analysis: Figure 1a

V. Does the spherical (or oval) particle have a smooth or irregular surface?

Smooth	6
Irregular/granular	9

VI. This is possibly a plastic sphere. What size is the sphere?

>1 mm	7
100 μm–1 mm	8

DOI: 10.1177/0003702820930733

VII. This may be classified as an oval pre-production resin pellet pending confirmatory analysis: Fig 1b

VIII. This may be classified as a pre-production plastic bead pending confirmatory analysis: Fig 1c

** Organic and inorganic alternatives include pearl, fisheye, sediment grain. phytoplankton shells, foraminifera, plant seed or seed casing. Further characterization may be required.*

IX. This is possibly a plastic grain: Fig 1d

This particle should be squeezed to see if it crumbles or crunches

** Organic and inorganic alternatives include pearl, fisheye, sediment grain, phytoplankton shells, foraminifera, plant seed or seed casing. Further characterization may be required.*

X. Does the particle sink in NaCl?

No	11
Yes	28

XI. Record as a suspected microplastic fragment pending confirmatory analysis. What type of edges does it have?

Fragment has angular edges	12
Fragment is rounded or has curved edges	13

XII. This suspected fragment could be further classified as either:

An angular fragment	Fig 1e
A sub-angular fragment	Fig 1f

XIII. This suspected fragment could be further classified as either:

A rounded fragment	Fig 1g
A sub-rounded fragment	Fig 1h

XIV. The overall shape of this malleable particles is:

DOI: 10.1177/0003702820930733

Clearly defined and spherical 15

Fragmented or fibrous (composed of single or multiple filaments) 18

XV. When pressure is applied how does the particle react?

Particle “pops” 16

Particle squashes but returns to original shape 17

XVI. This may be classified as a plastic bead pending confirmatory analysis: Fig.1i

** Organic alternatives include algae, seeds. Further characterization may be required.*

XVII. This may be classified as an EPS bead pending confirmatory analysis: Fig. 1j

XVIII. The particle morphology can be described as:

Fragmented 19

Fibrous, and composed of a single or multiple filaments 22

XIX. When the particle is manipulated and pressed:

The particle squashes and reforms 20

The particle can be bent/folded but is not soft 21

XX. This may be classified as a foam fragment pending confirmatory analysis: Fig 1k

XXI. This may be classified as a film pending confirmatory analysis: Fig 1l

XXII. In general, is each fiber homogeneously colored with an even thickness along the length?

Yes 23

No 26

XXIII. Does the filament display a cellular structure?

Yes 27

No 24

DOI: 10.1177/0003702820930733

XXIV. Does it have natural bending (ribbon like folding)?

No 25

Yes 26

XXV. This is possibly a plastic or anthropogenic fiber. Is it

A single fiber Fig 1m

** Organic alternatives include algae, natural fibers, regenerated fibers, plant fibers, macroinvertebrate fragments (e.g., antennae, legs). Further characterization may be required.*

A bundle of fibers Fig 1n

** Organic alternatives include algae, natural fibers, regenerated fibers, plant fibers. Further characterization may be required.*

XXVI. This fiber may be synthetic or organic. Classify as unknown. Further confirmatory steps are required.

XXVII. This could be algae. Refer to exclusion parameters in the main document.

XXVIII. This could be a sediment grain. Refer to exclusion parameters in the main document.

Excluding Non-Anthropogenic Items

The key included here is by no means a guarantee that the resulting particles are indeed plastics, and there are numerous materials of natural and manufactured origin that are regularly reported as a source of confusion. Caution and characterization should be applied at the discretion of the researcher. In the following sections we discuss how to separate commonly confused particles of non-plastic, mineral and organic construction based on visual identification using color, appearance, and feel as indicators.

Sediment Grains. As identified above, most sediment grains have a density of approximately 2.6 g cm^{-3} and will therefore sink when placed in low-density solutions, e.g., water, NaCl, Na(I), zinc chloride ($ZnCl_2$). Despite this, there is a possibility for sediment grains to form a composite particle or become "rafted" along with lighter material. This can result in mineral particles floating out density separation-based procedures. Therefore,

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sediment grains may be transferred to the final samples. To verify potential sediment grains, researchers are encouraged to test the properties of the particle. Sediment particles generally crunch or are gritty when handled with forceps. If there is no crunch, and the particle exhibits elasticity, it is not likely to be a sediment particle. Visual pre-selection should aim to exclude all large mineral grains if further chemical characterization is planned using attenuated total reflectance (ATR) FT-IR as the hardness of sand or gravel can fatally damage the coating of ATR crystals.

Organic Material. There are many types of organic material that may be present in the final sample after processing; many of these may be excluded depending on the initial sample type. One of the most commonly misidentified plastics are algae, which are easily fragmented and display distinct patterns of cells. One of the primary techniques by which organic material may be identified is through the presence of a visible cellular structure, not visible in many plastics (care should be taken not to confuse with foams and expanded polymers). Cellular structures are typically visible under a light microscope.

Similar to algal strands, clear cellulosic fibers may possess characteristic cellular structures visible with high resolution light microscopes. Cellulosic fibers may also exhibit spiny projections similar in appearance to branching plant roots (resembling a Y, Figure 2) projecting from the main fiber body, not to be confused with fiber fraying which is more regular in appearance. Cellulosic fibers also tend to break more easily upon contact, whereas anthropogenic fibers are more resistant to breakage.[37] Cellulosic fibers may also have a rough surface texture containing multiple pits relative to the often-smooth surface of anthropogenic clear fibers. Clear, cellulosic fibers may have tapered ends, and uneven thickness throughout the main fiber body.

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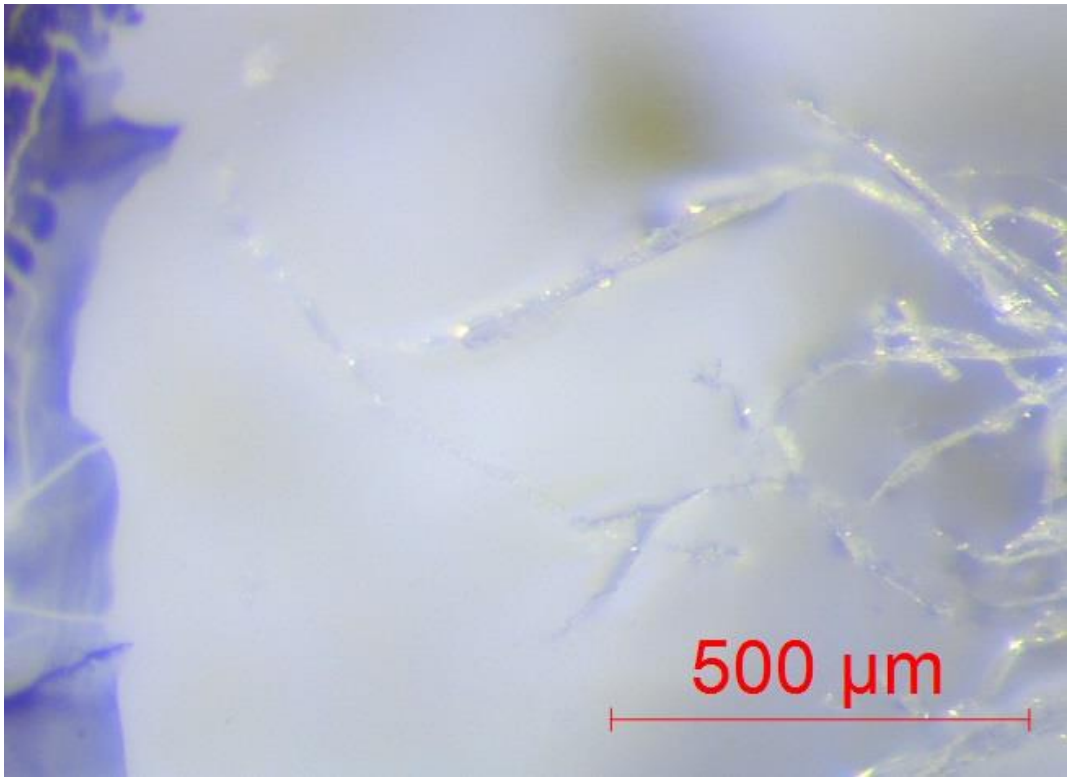

Figure 2. A cellulose fiber bundle pulled from a Kimwipe displaying the spiny projections (Y-shaped), rough surface texture and uneven thickness that are characteristic of cellulosic fibers.

In some cases, chitinous material is not fully removed during processing, and depending on the organisms these particles are derived from, they may resemble plastic material. However, in some cases chitinous remains are easily broken. Those that do not easily break but have a hard and shiny texture (e.g., beetle carapace), similar to plastics, should be prioritized for further characterization.

Seeds, eggs, eyes, eye lenses, and other spherical organic particles are broken when squeezed and may reveal a soft interior. The centers can sometimes resemble plastic films with a pliable texture which can be confusing if the exterior is missing. Fisheyes and squid lenses will break when pressure is applied, and their internal structure has several layers which can flake off. They have been misidentified in some studies (e.g., Peters et al.[51]). Lastly, pearls may be misidentified due to their smooth surface structure. However, on close inspection pearls have a pitted surface texture and will break/crumble when pressure is applied.

Salt Fragments/Crystals. When density solutions are used, salt fragments may crystallize on filter papers during drying. They may present to the observer like angular fragments with a gloss-like surface texture, most commonly clear or white. To eliminate

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these from anthropogenic particles they can be gently pressed with forceps or dissecting needles. Salt crystals break easily and generally have a visible crystalline structure. Furthermore, thoroughly washing filter papers with laboratory water following filtration can help to eliminate this issue.

Case Study

Visual identification using the proposed morphological categories was used in a case study to identify suspected microplastics from several Laurentian Great Lakes fish caught in 2015 and 2016 (n= 47) as part of a larger microplastics study at the University of Toronto, Ontario, Canada. Gastrointestinal tracts were dissected and digested using an alkaline digestion method adapted from Foekema et al.[52] The remaining particles were sieved to >125 μm and filtered onto 47 mm diameter, 10 μm polycarbonate filters. Filters were examined under a dissecting microscope (Leica S8 APO Stereozoom; Leica Microsystems Canada, Inc., Canada) at 10–80x magnification. A subset of suspected microplastics (n_{MP}=150) were manually extracted from filters and were placed in petri dishes lined with double-sided tape mounted on transparent film for further analysis. Extracted particles included a variety of morphologies and colors were picked out to assess accuracy in visual identification and classification using the proposed morphological categories. Some microparticles that were suspected to be non-synthetic and non-anthropogenic (i.e., suspected non-plastic) were also mounted on double-sided tape. Suspected non-plastics of a variety of colors and morphologies were extracted to assess the potential for false negative identification (i.e., particles were not counted as suspected microplastics but were in fact microplastic or anthropogenic) (non-MP=50).

Extracted particles were analyzed via spectroscopy to confirm polymeric composition. Infrared spectra were acquired using a Bruker ALPHA II Platinum Attenuated Total Reference FT-IR with a diamond internal reference (Bruker Ltd., Canada) and OPUS software (v.7.8.44). The range for IR spectra was set to 4000 to 400 cm^{-1} and the resolution was 4 cm^{-1}. The sample scan time and background scan time were both 24 scans. Infrared spectra were matched using OPUS software and associated reference libraries. Raman spectroscopy was performed using a Horiba Raman XxploRA PLUS confocal Raman microscope operated with LabSpec6 (v.6.5.1.24) and a charge-coupled device (CCD) detector (–60°C, 1024 x 256 pixels). Raman spectra were acquired with a 100X LWD objective in the range of 100–3500 cm^{-1} using 785 nm (1.325 cm^{-1}/pixel; maximum laser power = 20.2 mW) and 532 nm (3.252 cm^{-1}/pixel; maximum laser power = 11.2 mW) lasers. Parameters for

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spectral acquisition were determined using the application-based library.[53] Manual baseline correction and smoothing of Raman spectra was performed in LabSpec6 if necessary. Raman spectra were matched using Bio-Rad KnowItAll software with associated reference libraries and the SLoPP/SLoPP-E Raman libraries created at the University of Toronto.[53] Automatic corrections in Bio-Rad KnowItAll software may have been applied (baseline, vertical clipping, intensity distortion, horizontal offset, vertical offset, and Raman intensity distortion). Spectral matches to databases of reference spectra were assigned based on manual inspection of peak position and intensity, and by the Hit Quality Index. Particles were grouped by polymeric composition as "plastic", "anthropogenic (cellulosic)" (including modified cellulose and cellulose combined with pigments), "anthropogenic (synthetic)" including fillers and additives, associated with synthetic materials, e.g., plasticizers), "anthropogenic (unknown)" (including pigments where underlying polymers could not be detected), "natural" (cellulosic, other materials organic or inorganics), or "unknown" (no spectrum obtained, or no spectral match identified) (Tables S1 and S2, Supplemental Material).

Several plastic polymers were detected via spectroscopy from suspected microplastics (Figure 3a). Anthropogenic (cellulosic), polyethylene (PET), PET/polyester, and polypropylene polymers were frequently detected. Polymer verification via spectroscopy confirmed that only 9% of the particles were misidentified as anthropogenic and were actually natural cellulosic, other organic materials (e.g., hair) or natural inorganic particles (e.g., minerals) (Figure 3b). An additional 4% could not be identified due to photodegradation, fluorescence, or a lack of conclusive match in the Raman and FT-IR spectral databases. The remaining 87% were correctly identified as plastic or anthropogenic. "Anthropogenic" includes particles with a pigment and cellulosic polymer, a synthetic base, and an unknown base where only the dye is detected. It is difficult or impossible to identify polymeric composition of some particles via Raman spectroscopy when pigments mask peaks associated with the polymer,[54] which is a common issue in Raman spectroscopy of microplastics.[53] Particularly in the case of fibers, it can be difficult to distinguish visually and using spectroscopy whether a particle is plastic or anthropogenic with a non-plastic material type.[53] However, these particles are still of importance when determining the load of microparticles in the environment that are of anthropogenic origin.

Approximately 82% of suspected non-plastic particles were confirmed to be natural (Figure 3c). Of the remaining particles, 10% were incorrectly identified as non-plastic and 8% could not be identified. The majority of suspected non-plastics were identified as natural

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polymers (e.g., cellulosic, other organic materials and inorganics). While it is clear that some particles are missed during visual identification, the rate of false negative identification is relatively low. Overall, the level of accuracy in identifying plastic and other anthropogenic particles is relatively high compared to previously reported figures.[14,54]

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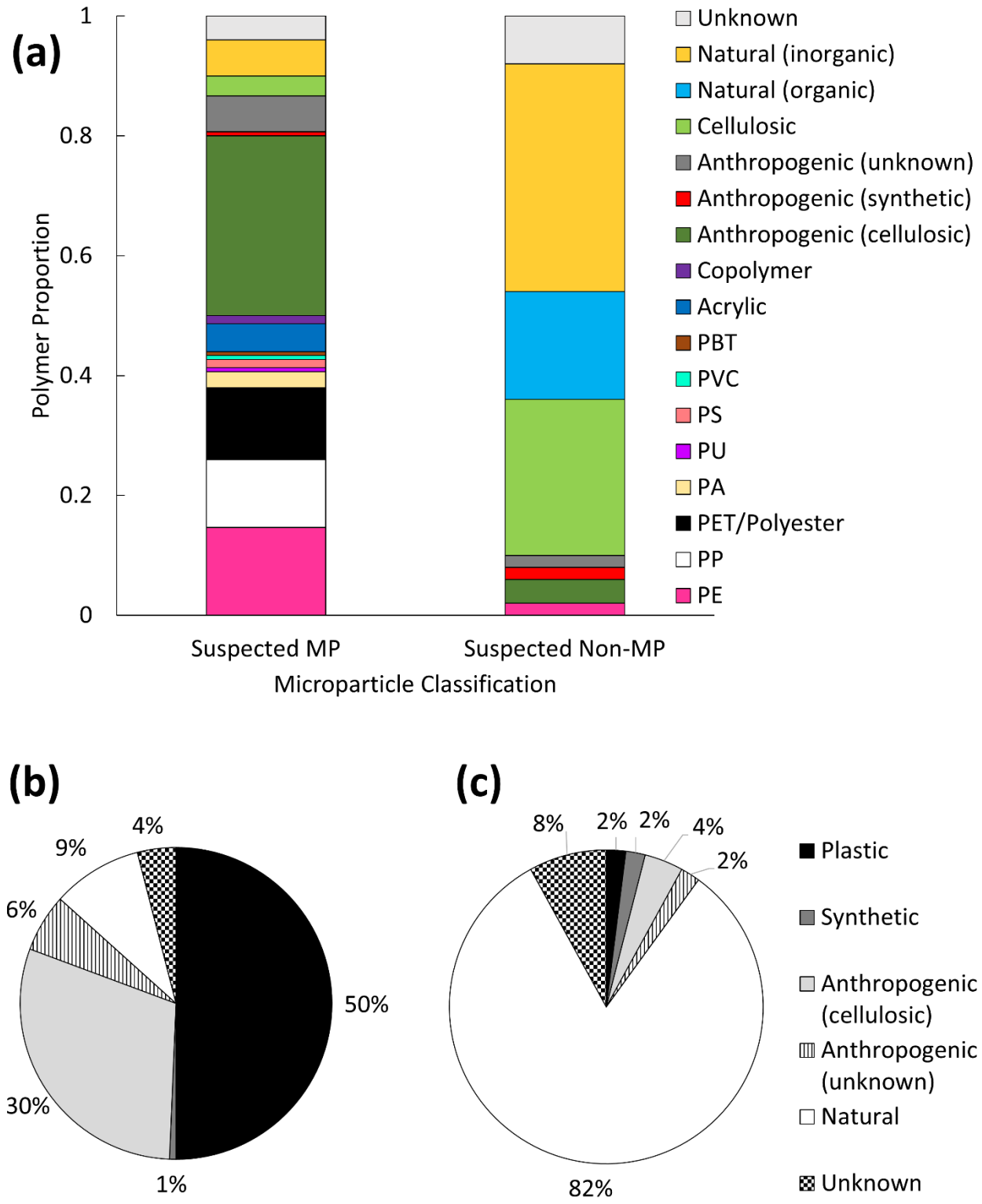

Figure 3. Microparticles in 47 fish gastrointestinal tracts were classified using the proposed morphological categories. (a) Extracted microparticles were analyzed using Raman and FT-IR spectroscopy to determine polymeric composition. Particles were sorted into polymer

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groups based on spectroscopic identification for (b) suspected microplastics and (c) suspected non-microplastics.

Discussion

Guides to aid in visual microplastic identification are already available in the published literature (e.g., Hidalgo-Ruz et al.,[11] Rochman et al.,[37] Lusher et al.,[43] Kroon et al.,[55] and Karlsson et al.[56]); however, they typically lack breadth and depth, or are confined to literature reviews, a short methodology section or the supplementary materials. Here, we present a thorough explanation of steps to classify microplastics, whilst highlighting potential for misidentification and introducing steps to mitigate erroneously classification. A summary of the recommendations discussed throughout are presented in Table II.

Table II. Recommendations to aid in the visual identification of microplastics.

Category	Recommendation
Particle morphology	
Particle size	Researchers should explicitly state the method they follow to report size: Longest dimension, Feret's diameter, two-dimensional surface area ,or three-dimensional volume. Researchers should include at the very minimum the size range of particles they investigated, from the smallest (defined by the sampling and analytical methodology) to the largest particle. Where possible all recorded sizes, and subsequent size categories used should be reported.
Particle shape	Particle shapes can be grouped in to three broad categories, which can be differentiated further. Bead (sphere, hemisphere, grain, nurdle, EPS), fiber (singular fiber, fiber bundle), fragment (foam, film, angular/sub-angular, rounded/sub-rounded) Researchers should use the lowest level of categorization possible to aid comparisons between studies. Specifically, all fibers should be fully characterized, where this is not possible, the highest proportion should be analyzed, and published data should be explicit about the

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	size of the subsample and the percentage of which were identified as plastic.
Particle texture	Particle texture can be explained using a description of surface roughness. It is recommended that researchers attempt to characterize surface roughness where possible.
Optical properties	
Particle color	Particle colors should be reported to the level of secondary colors (red, orange, yellow, green, blue, violet) as well as including black, white, and clear.
Birefringence	Researchers should determine the occurrence and apparent strength of the observed refractive index at n‖ and n†, achieved by observing the fiber with the polarization plane oriented parallel (n‖) and perpendicular (n†) to the longest axis. For fibers displaying two refractive indices, birefringence should be calculated as the numerical difference in refractive indices for a fiber, achieved via the formula: n‖ – n†.
Physical behavior	
Elasticity	It is recommended that researchers physically manipulate particles (>200 μm) to aid in visual identification. Squeezing can test malleability, elasticity and crunching.
Density	Particle suspected of being organic can be subject to a secondary density assessment.
Reporting requirements	Researchers are recommended to be explicit on the percentage of particles further characterized using spectral instruments. Where particles not explicitly confirmed as plastic it is recommended that the term anthropogenic microparticles (AMPs) is introduced.
Confirmatory analysis	A proportion of all particle >500 μm should be subjected to further characterization (FT-IR/Raman etc.) where possible. 100% characterization is required for particles <200 μm

Limitations of Visual Identification

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As identified above, visual classification alone is insufficient to determine microplastic abundance. Fibers represent a particular challenge for visual-based identification methods. They are difficult to separate from non-plastic synthetic (e.g., cellulosic fibers) and natural fibers (e.g., cotton or wool) and in some cases also fibrous organic material (e.g., plant fibers or short fragments of chitin derived from macroinvertebrates). Reliable enumeration is also affected by the high proportion of laboratory atmospheric contamination composed of fiber particles. Figure 4 demonstrates the difficulties in visually identifying plastic fibers. All fibers depicted would pass the criteria identified in the visual identification key here, as they all exhibit homogeneous thickness and color, are shiny, and do not present any cellular structure. They are also cleanly cut at the ends. Despite this, only three particles were confirmed to be plastic using FT-IR (#3, 4, 7; Figure 4). Particles 2 and 15 appear to most closely resemble microplastic fibers due to their high degree of shine and structure; however, these were identified as wool and cellulose, respectively.

It is recommended that, all fibers identified in environmental samples should be characterized; where this is not possible, the highest proportion should be analyzed, and published data should be explicit about the size of the subsample and the percentage of which were identified as plastic. Where complete analysis is achievable, a subset of fibrous particles that have been visually assessed as likely to be of natural origin should also be chemically characterized. The number of false negatives should be used to identify the potential for an underestimation of plastic fiber content. Where fibers (and other particles) which are not explicitly confirmed as plastic the term anthropogenic microparticles (AMPs) is sometimes encouraged.[57]

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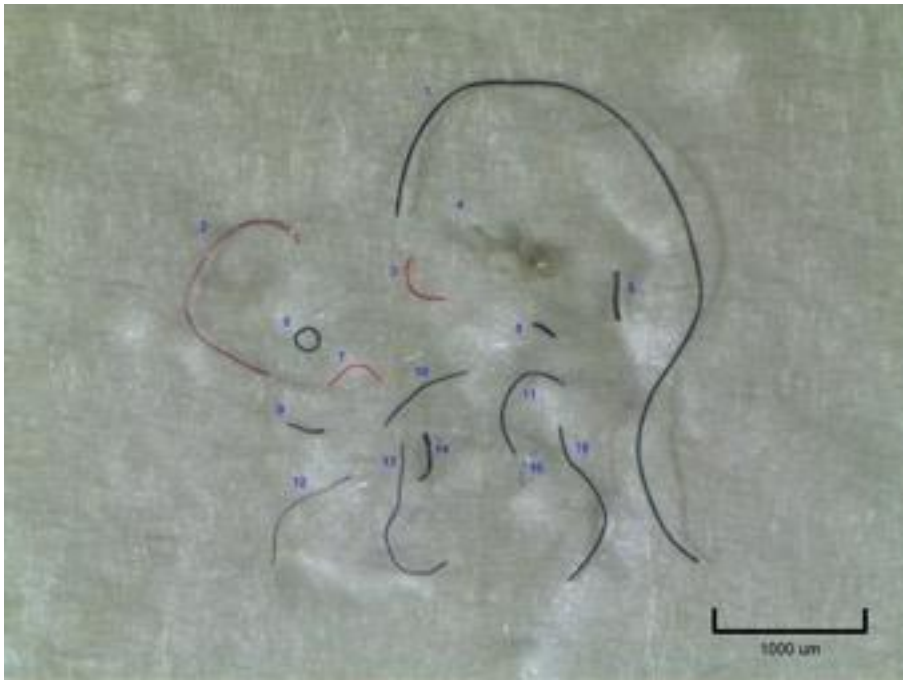

Figure 4. Suspected microplastic particles isolated from a freshwater sample collected in Norway. Chemical characterization using FT-IR: 1. Wool fiber; 2: wool fiber; 3: polyester fiber; 4: polypropylene fiber; 5: wool fiber; 6 wool fiber; 7: acrylic fiber; 8: wool fiber; 9: wool fiber; 10: wool fiber; 11: wool fiber; 12: wool fiber; 13: wool fiber; 14: wool fiber; 15: cellulose fiber; 16: wool fiber.

In addition to the challenges posed by specific particle classes, particle size limits the effectiveness of visual identification. When visual classification alone is available, a lower size limit of 500 µm is recommended;[33] however, further chemical characterization should be included whenever possible to verify visual findings. Furthermore, we recommend using 200 µm as a tentative lower size limit for some particles based on the requirements for visually assessing texture and appearance and physically handling particles. Lastly, we recommend increasing the proportion of particles that receive further chemical characterization as the lower size limit decreases, with 100% characterization recommended for particles <200 µm.

In an ideal world, thorough classification and characterization should be conducted on all suspected microplastics. Where this is not possible, as identified above, researchers should make specific reference to the proportion of the total visually identified particles that were submitted for chemical characterization, particle classes (fibers, beads, etc.), and size fractions. Thorough testing should include examples of all microplastic morphological categories observed to highlight potential observer bias, and tests should be performed prior to data analysis of microplastic concentrations to allow for appropriate data correction, such as removing items confirmed to be non-plastic.

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Recommendations based on literature reviews have been published outlining the minimum proportion (10%) of particles derived from biota and water samples that are to be subjected to further chemical characterization based on particle size classes.[58,59] Whereas in a recent assessment of microplastic levels in Dutch rivers, the levels of uncertainty were acceptable when analyzing 50% of a filter during chemical mapping, and when identifying at least a subset of 50 individual particles with attenuated total reflection (ATR) FT-IR.[60.] Whilst complete chemical characterization of all microplastic particles is the ultimate aim, the harmonized visual identification guide presented here, coupled with a thorough description of the analytical procedure, will improve the quality of studies that continue to rely on a visual component for microplastic analysis.

Value of Visual-Based Identification Methods

As identified above, semi-automated spectroscopic imaging techniques help to reduce the need for any visual pre-selection; however, visual-based methods may still be required to improve the efficiency of analysis. For example, FT-IR and Raman imaging approaches work best with a narrower focal range, where larger particles should be removed in order to effectively analyze the smallest fraction (i.e., small and large particles will not be visible at the same level of focus due to the aperture used) and enable higher spatial resolution. Larger particles may also be removed to prevent physically obscuring smaller particles that may be concealed beneath them on the filter surface. The presence of large particles may also lead to observer bias. For example, it may appear that all particles on a filter surface have been detected with a lower magnification objective (e.g., 10X) as smaller particles are not visible and the use of higher magnification objectives may be limited by particle size and proximity to the objective lens. Additionally, different spectroscopic methods have different size-limitations, and those involving physical handling of suspected microplastics require some visual identification prior to polymer verification. Cabernard et al.[61] visually identified and manually extracted all suspected microplastic particles >500 μm from processed samples and further analyzed them using ATR FT-IR where particles were physically placed on the ATR FT-IR crystal. Smaller size fractions were analyzed using alternative methods (μ-Raman spectroscopy and FT-IR imaging) that did not require particles to be physically removed from the filters and placed onto the instrument. Furthermore, automated imaging techniques may not effectively characterize fiber particles based on their irregular shape and propensity to curl away from dried filters as they bend and twist. Hence, visual appraisal of samples may

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be required before submitting them for imaging, and larger fibers removed for further characterization through different means.

Visual identification provides morphological information to researchers, which is important when inferring risks to ecosystems. Not all microplastics are alike, and morphology is a useful characteristic when considering microplastics as a diverse group of environmental contaminants.[37] For example, a meta-analysis of the effects of microplastics on aquatic organisms found that round particles were associated with negative effects on all four types of responses considered (growth, consumption, reproduction and survival), whereas fibrous or fragmented microplastics only negatively affected survival.[23] Other responses ranged from negative to neutral for fragmented and fibrous microplastics.[23] Another study demonstrated differing fate and effects of morphologies on grass shrimp (*Palaemonetes pugio*).[62] The number of fragments observed in shrimp guts was significantly higher than spheres and fibers, yet the opposite was observed in the gills.[62] All size fractions of fibers tested resulted in mortality for grass shrimp ranging from 35–55%, but this was not true for fragments or spheres.[62] While some studies have investigated morphologically linked effects, more is required as morphology is an important characteristic.

It has also been demonstrated that different morphologies behave differently in the environment. Physical properties, including shape, define particle behavior and fate (motion, residence time, biofouling celerity, etc.). For example, a PE film may remain in the euphotic zone of the Baltic Sea for three to four months, while a PE fiber may remain six to eight months, and a PE sphere may remain 10–15 years assuming the particles do not become fragmented.[21] The amount of time a particle remains in a particular environment will affect the amount of weathering, UV degradation, biofouling, and potential for exposure to organisms, further demonstrating the importance of morphological classification for ecological considerations. Morphology of microplastics is also important for policymakers trying to limit the use and generation of specific particle types. Certain morphologies have been cited in legislation, such as microbead bans for personal care and cosmetic products which have been put into place in several countries. Morphology and color can be used to infer sources[22] which may inform future policy decisions.

Conclusion

Developing our understanding of global microplastic pollution requires a holistic knowledge of the distribution of polymer types, their morphology, and potential effects. Whilst numerous methods have been developed to facilitate the characterization of microplastics,

their morphological classification has remained inconsistent. Additionally, the limitations of characterization and regular sub-sampling show clear need for improvement in visual sorting. The proposed protocol has been generated to provide researchers with a practical tool for the visual assessment of microplastics. Initial trials highlight its effectiveness in improving and streamlining the identification and categorization of suspected plastic particles. It should not be used as a replacement to polymer confirmation by chemical analytical methods, but as a tool to support research, especially when sample sizes are high and high throughput of analysis is required, such as monitoring regimes requiring fast results.

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Supplementary material

Is it or isn’t it: the importance of visual classification in microplastic characterization.

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Figure SI.1 Flow chart decision tree for the identification of potential microplastics in environmental samples

Table SI1. A group of 150 Raman spectra acquired from environmental particles that are visually identified as suspected microplastics consist of a variety of colors and morphologies. The best match is reported (Raman ID) as determined by Hit Quality Index, peak position and peak intensity. The polymer groups are determined based on Raman ID.

Table SI2. Fifty Raman spectra were acquired from environmental particles that are visually identified as suspected non-plastic particles. The best match is reported (Raman ID) as determined by Hit Quality Index, peak position and peak intensity. The polymer groups are determined based on Raman ID.

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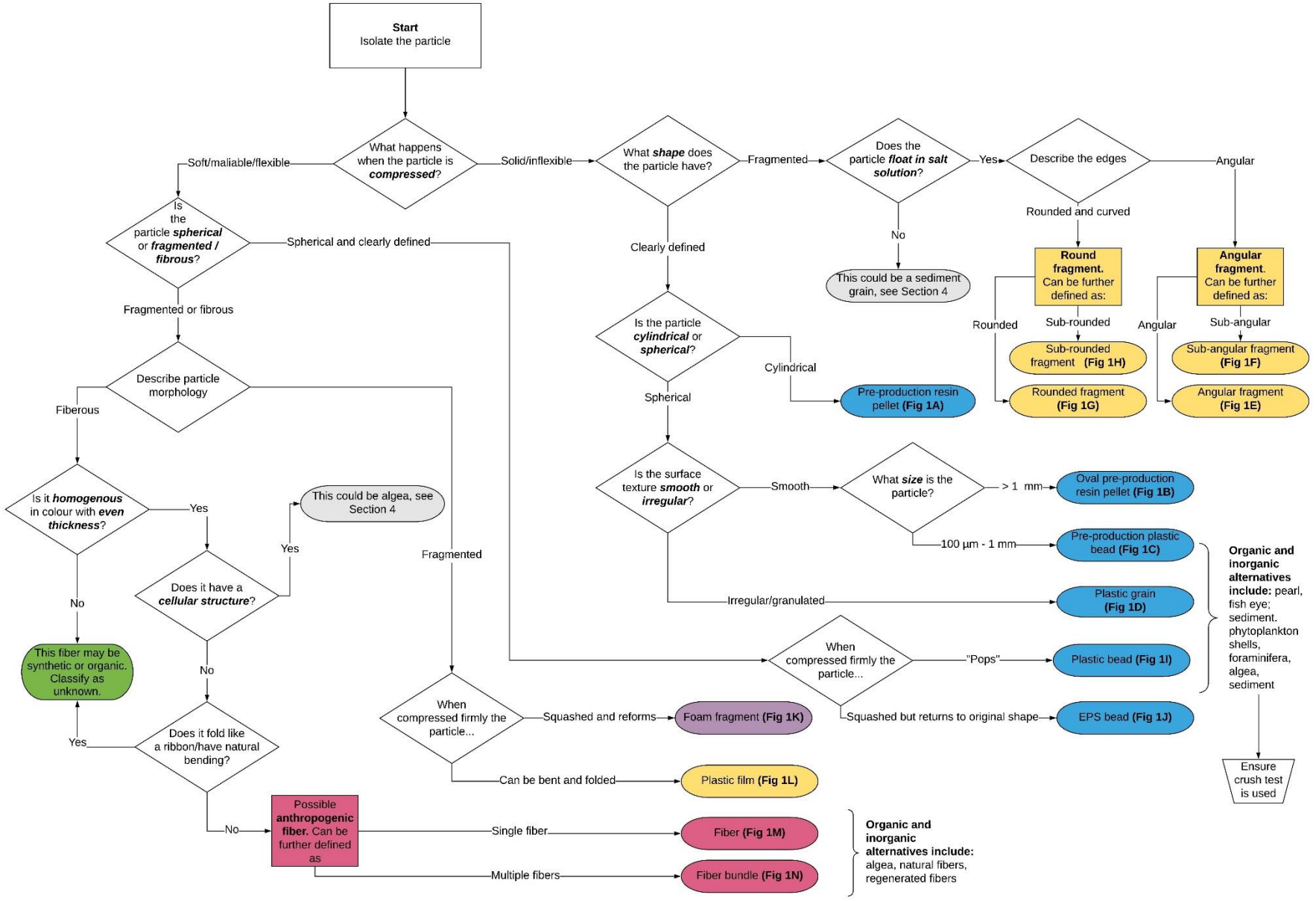

Figure SI.1 Flow chart decision tree for the identification of potential microplastics in environmental samples

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Table SI1. A group of 150 Raman spectra acquired from environmental particles that are visually identified as suspected microplastics consist of a variety of colors and morphologies. The best match is reported (Raman ID) as determined by Hit Quality Index, peak position and peak intensity. The polymer groups are determined based on Raman ID.

Particle ID	Category	Color	Raman ID	Polymer Group
1	fiber	black	cotton 9. black fiber	Anthropogenic (cellulosic)
2	fiber	blue	magenta 1	Anthropogenic (unknown)
3	fragment	blue	PE 8. light blue film	PE
4	film	grey	PP	PP
5	fiber	red	cotton 8. red fiber	Anthropogenic (cellulosic)
6	film	blue	PE 8. light blue film	PE
7	fragment	blue	PE 8. light blue film	PE
8	fiber	black	cotton 9. black fiber	Anthropogenic (cellulosic)
9	fiber	blue	fluorescence	Unknown
10	fiber	red	cellulose acetate 3. pink fiber	Anthropogenic (cellulosic)
11	fiber	blue	indigo carmine	Anthropogenic (unknown)
12	fiber	yellow	cotton 7. orange fiber	Anthropogenic (cellulosic)
13	fiber	red	cotton 8. red fiber	Anthropogenic (cellulosic)
14	fiber	black	cotton 9. black fiber	Anthropogenic (cellulosic)
15	fragment	blue	PE 20. white microsphere	PE
16	fiber	red	cotton 2. pink fiber	Anthropogenic (cellulosic)
17	fragment	blue	indigo carmine + PET 1. red bottle (composite)	PET/Polyester
18	fiber	black	cotton 1. grey fiber	Anthropogenic (cellulosic)
19	fiber	green	dyed cellulose 3. green fiber	Anthropogenic (cellulosic)
20	fiber	black	polyacrylonitrile + eriochrom blue SE (composite)	Acrylic
21	fiber	blue	acrylic 9. yellow fiber	Acrylic
22	fiber	black	cotton 9. black fiber	Anthropogenic (cellulosic)

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23	fiber	blue	indigo carmine	Anthropogenic (unknown)
24	fiber	black	indigo carmine	Anthropogenic (unknown)
25	fiber	black	cotton 9. black fiber	Anthropogenic (cellulosic)
26	fragment	black	polyester	PET/Polyester
27	fragment	blue	silica	Natural (inorganic)
28	fragment	clear	PP 10. brown fiber	PP
29	fiber	blue	dyed cellulose 1. red fiber	Anthropogenic (cellulosic)
30	fragment	blue	PE 7. blue fragment	PE
31	fragment	blue	PE 7. blue fragment	PE
32	fiber	red	cotton 8. red fiber	Anthropogenic (cellulosic)
33	film	blue	PE 22. blue foam	PE
34	fragment	blue	PE 8. light blue film	PE
35	bead	purple	PE 9. clear cling wrap	PE
36	fiber	clear	polyacrylonitrile	Acrylic
37	fiber	clear	polyamide 2. white pellet	PA
38	fragment	clear	PP 8. white bottle cap	PP
39	fragment	clear	orthoclase	Natural (inorganic)
40	fragment	clear	PS	PS
41	fragment	blue	PE 4. orange bin	PE
42	fiber	blue	polyester 10. dark blue fiber	PET/Polyester
43	fiber	blue	ivory black	Unknown
44	fragment	blue	PE 9. light blue film	PE
45	fiber	clear	cotton 12a. Grey fiber	Cellulosic
46	fragment	clear	polyester	PET/Polyester
47	fiber	clear	cotton 3a. Yellow fiber	Cellulosic
48	fiber	blue	indigo	Anthropogenic (unknown)
49	fiber	blue	cotton 9. black fiber	Anthropogenic (cellulosic)
50	fiber	blue	polyester 11. blue fiber	PET/Polyester
51	fiber	black	cotton 9. black fiber	Anthropogenic (cellulosic)
52	fiber	black	cotton 9. black fiber	Anthropogenic (cellulosic)
53	fiber	pink	cotton 8. red fiber	Anthropogenic (cellulosic)
54	fiber	clear	no peaks	Unknown
55	fragment	clear	smalt	Natural (inorganic)

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56	fiber	blue	indigo carmine	Anthropogenic (unknown)
57	fiber	blue	cotton 9. black fiber	Anthropogenic (cellulosic)
58	fiber	black	cotton 9. black fiber	Anthropogenic (cellulosic)
59	fragment	black	no peaks	Unknown
60	fragment	white	chalk	Natural (inorganic)
61	fragment	clear	PP 15. clear cling wrap	PP
62	fiber	clear	cotton 16. white lab coat	Cellulosic
63	fiber	red	cotton 8. red fiber	Anthropogenic (cellulosic)
64	fiber	clear	cotton 9. black fiber	Anthropogenic (cellulosic)
65	fragment	blue	indigo + polyester 7. pink fiber (composite)	PET/Polyester
66	fragment	white	cadmium indium sulfide	Unknown
67	fragment	white	hostopen violet	Anthropogenic (unknown)
68	fiber	clear	polyester 11. blue fiber	PET/Polyester
69	fiber	blue	cotton 9. black fiber	Anthropogenic (cellulosic)
70	fiber	black	cotton 9. black fiber	Anthropogenic (cellulosic)
71	fiber	blue	cotton 9. black fiber	Anthropogenic (cellulosic)
72	fiber	blue	acrylic 10. pink fiber	Acrylic
73	fragment	blue	PP 15. green fiber	PP
74	fiber	clear	cotton 9. black fiber	Anthropogenic (cellulosic)
75	fiber	clear	dyed cellulose 1. red fiber	Anthropogenic (cellulosic)
76	fragment	clear	bytownite	Natural (inorganic)
77	fragment	red	PP 15. clear cling wrap	PP
78	film	blue	PE 8. light blue film	PE
79	fragment	blue	PP 15. green fiber	PP
80	fragment	green	PET 13. clear plastic sheet	PET/Polyester
81	fragment	red	PP 18. purple foam	PP
82	fragment	black	PBT 1. pink fragment	PBT
83	fiber	blue	indigo carmine	Anthropogenic (unknown)
84	fragment	clear	polyacrolein	Anthropogenic (synthetic)

DOI; 10.1177/0003702820930733

85	fragment	blue	PE 21. blue film	PE
86	fragment	clear	polyamide resin	PA
87	fiber	black	cotton 9. black fiber	Anthropogenic (cellulosic)
88	fragment	black	cotton 9. black fiber	Anthropogenic (cellulosic)
89	film	clear	PP 15. clear cling wrap	PP
90	fragment	clear	ceric oxide	Natural (inorganic)
91	fragment	clear	PP 15. clear cling wrap	PP
92	fiber	clear	cotton 12a. Grey fiber	Cellulosic
93	fiber	blue	cotton 11a. Brown fiber	Anthropogenic (cellulosic)
94	fiber	clear	acrylic 1. green yarn	Acrylic
95	fragment	green	malachite	Natural (inorganic)
96	fragment	blue	ethylene/propylene/diene terpolymer	Copolymer
97	fiber	clear	polyester 3. dark blue fiber	PET/Polyester
98	fiber	blue	cotton 9. black fiber	Anthropogenic (cellulosic)
99	fiber	blue	cotton 11. brown fiber	Anthropogenic (cellulosic)
100	fiber	blue	polyester 10. dark blue fiber	PET/Polyester
101	fiber	blue	cotton 9. black fiber	Anthropogenic (cellulosic)
102	fragment	clear	PP 21. white film	PP
103	fiber	clear	cotton 12a. Grey fiber	Cellulosic
104	fragment	clear	PET	PET/Polyester
105	fragment	clear	PET	PET/Polyester
106	fiber	clear	nylon	PA
107	fiber bundle	clear	PP	PP
108	fragment	blue	PE	PE
109	fragment	blue	PS	PS
110	fragment	black	colour blend + PE	PE
111	film	clear	PE	PE
112	fiber	clear	PP	PP
113	film	clear	PE + PP	Copolymer
114	fragment	white	PE	PE
115	fragment	clear	PE	PE
116	film	clear	PE	PE
117	fragment	black	PE	PE
118	fragment	yellow	PP	PP
119	fiber	black	nylon	PA

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120	fragment	pink	PVC	PVC
121	fiber	blue	polyester 3. dark blue fiber	PET/Polyester
122	fiber	blue	indigo carmine + polyacrylonitrile (composite)	Acrylic
123	fiber	pink	cotton 1. red fiber	Anthropogenic (cellulosic)
124	fiber	blue	polyester 3. dark blue fiber	PET/Polyester
125	fiber	pink	PET 1. red bottle	PET/Polyester
126	fragment	clear	quartz	Natural (inorganic)
127	fiber	clear	polyester 1. clear fiber	PET/Polyester
128	fiber	clear	carbon + PET 1. red bottle (composite)	PET/Polyester
129	fiber	black	cotton 9. black fiber	Anthropogenic (cellulosic)
130	fragment	blue	PP 15. green fiber	PP
131	fiber	pink	PP 15. clear cling wrap	PP
132	fiber	blue	cellulose acetate 4. white fiber	Anthropogenic (cellulosic)
133	fiber	blue	cotton 9. black fiber	Anthropogenic (cellulosic)
134	fiber	black	cotton 9. black fiber	Anthropogenic (cellulosic)
135	fiber	pink	PU 1. black fiber	PU
136	fiber	black	cotton 9. black fiber	Anthropogenic (cellulosic)
137	fiber	clear	cellulose acetate sorbate	Anthropogenic (cellulosic)
138	film	black	no peaks	Unknown
139	fiber	blue	acrylic 2. pink fiber bundle	Acrylic
140	fiber	black	polyester 10. black fiber	PET/Polyester
141	fragment	blue	PE 8. light blue film	PE
142	fiber	red	cotton 8. red fiber	Anthropogenic (cellulosic)
143	fiber	clear	PE	PE
144	fiber	pink	dyed cellulose 2. pink fiber	Anthropogenic (cellulosic)
145	fragment	clear	orthoclase	Natural (inorganic)
146	fiber	blue	cotton 9. black fiber	Anthropogenic (cellulosic)
147	fiber	blue	cotton 9. black fiber	Anthropogenic (cellulosic)
148	fragment	blue	PP 15. green fiber	PP

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149	fiber	clear	cellulose acetate sorbate	Anthropogenic (cellulosic)
150	fiber	blue	indigo	Anthropogenic (unknown)

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Particle ID	Category	Color	Raman ID	Polymer Group
1	fragment	white	mucopolysaccarides	Natural (organic)
2	fragment	white	cellulose propionate	Cellulosic
3	fragment	white	mucopolysaccarides	Natural (organic)
4	fiber	clear	cotton 12a. Grey fiber	Cellulosic
5	fragment	yellow	bone white	Natural (organic)
6	fiber	clear	cellulose	Cellulosic
7	fragment	black	obsidian	Natural (inorganic)
8	fragment	white	no peaks	Unknown
9	fiber	white	cellulose	Cellulosic
10	fragment	clear	quartz	Natural (inorganic)
11	fragment	black	schorl	Natural (inorganic)
12	fragment	clear	quartz	Natural (inorganic)
13	fiber	clear	cellulose	Cellulosic
14	fragment	brown	forsterite	Natural (inorganic)
15	fragment	orange	dentin	Natural (organic)
16	fragment	yellow	bone white	Natural (organic)
17	fragment	clear	quartz	Natural (inorganic)
18	fiber	clear	lysozyme human	Natural (organic)
19	fragment	white	calcium carbonate	Natural (inorganic)
20	fiber	clear	dyed cellulose 1. red fiber	Anthropogenic (cellulosic)
21	fragment	white	siderite	Natural (inorganic)
22	fragment	clear	cumengite	Natural (inorganic)
23	fragment	clear	carbon	Natural (inorganic)
24	fragment	clear	amethyst	Natural (inorganic)
25	fragment	clear	quartz	Natural (inorganic)
26	fragment	black	coral	Natural (organic)
27	fragment	white	horn of stag	Natural (organic)
28	fragment	black	ivory black	Unknown
29	fragment	orange	van dyke brown	Unknown

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30	fragment	white	aragonite	Natural (inorganic)
31	fiber	clear	cotton 16. white lab coat	Cellulosic
32	fiber	clear	cellulose	Cellulosic
33	fragment	clear	bytownite	Natural (inorganic)
34	fiber	clear	cellulose	Cellulosic
35	fiber	clear	cellulose	Cellulosic
36	fragment	clear	sanidine	Natural (inorganic)
37	fragment	clear	labradorite	Natural (inorganic)
38	fiber	clear	cotton 3. yellow fiber	Cellulosic
39	fiber	clear	hair	Natural (organic)
40	fragment	clear	quartz	Natural (inorganic)
41	fragment	clear	albite	Natural (inorganic)
42	fragment	clear	quartz	Natural (inorganic)
43	film	black	cotton 9. black fiber	Anthropogenic (cellulosic)
44	fragment	pink	burned	Unknown
45	fragment	clear	alpha-cyclodextrin	Anthropogenic (unknown)
46	fragment	clear	Amojell	Anthropogenic (synthetic)
47	fragment	clear	PE 21. blue film	PE
48	fiber	clear	cellulose	Cellulosic
49	fiber	clear	cellulose	Cellulosic
50	fiber	clear	cotton 9. black fiber	Anthropogenic (cellulosic)